

AMENDMENTS TO THE SPECIFICATION:

Please replace the title with the following:

METHOD OF INDUCING THE DIFFERENTIATION OF STEM CELLS INTO
CARDIOMYOCYTES

Please replace paragraph [0006] with the following replacement paragraph:

Pluripotent stem cells are defined as cells which are capable of indefinite or long-term cell proliferation while remaining in an undifferentiated state ~~in an~~ in an in vitro culture, which retain normal karyotypes, and which have the ability to differentiate into all of three germ layers (ectoderm, mesoderm and endoderm). At present, the three well-known pluripotent stem cells are embryonic stem cells (ES cells) derived from early-stage embryos, embryonic germ cells (EG cells) derived from primordial germ cells at the embryonic stage, and multipotent adult progenitor cells (MAPC) isolated from adult bone marrow.

Please replace paragraph [0010] with the following replacement paragraph:

Kehat et al (See Non-Patent Document 8) and ~~Chunhui Xu~~ Chunhui Xu et al (See Patent Document 4; Non-Patent Document 9) have reported that human ES cells can differentiate into cardiomyocytes in vitro, as mouse ES cells can do. According to these reports, human ES cells-derived cardiomyocytes which have been induced to differentiate from human ES cells not only have the ability to beat spontaneously but also express and produce myocardial-specific proteins such as myosin heavy and light chains, alpha-actinin, troponin I and atrial natriuretic peptide (ANP) and myocardial-specific transcription factors such as GATA-4, Nkx2.5, MEF-2c and the like, and from microanatomical observation and electrophysiological analysis it appears that they retain the properties of immature cardiomyocytes at the fetal stage, and could be used for regenerative therapy.

Please replace paragraph [0011] with the following replacement paragraph:

However, one serious problem remains to be elucidated to use pluripotent stem cells-derived cardiomyocytes for cell transplantation therapy and other purposes. When ~~[[EBS]]~~ EBs are formed from ES cells or EG cells by conventional methods, not only cardiomyocytes, but also other types of differentiated cells, such as blood cells, vascular cells, neural cells, intestinal cells, bone and cartilage cells and the like, are developed. Moreover, the proportion of cardiomyocytes in these differentiated cell population is not so high, only about 5 to 20% of the total.

Please amend the “Non-Patent Document 9” entry at page 9, line 14 as follows:

~~Chunhui Xu~~ Xu et al. Circ. Res. 91: ~~[[508]]~~ 501

Please amend the Figure 4 description at page 19, lines 8-11 as follows:

Figure 4 shows the results of ~~immunochemical~~ immunocytochemical staining of isolated cardiomyocytes from Noggin (+) group of EBs (derived from EB3 cells) on the 10th day of floating culture. a: sarcomeric myosin, b: troponin I, c: α -actinin, d: ANP

Please amend the Figure 5 description at page 19, lines 12-16 as follows:

Figure 5 shows the results of ~~immunochemical~~ immunocytochemical staining of cardiomyocytes in Noggin (+) and Noggin (-) groups of EBs (derived from EB3 cells) on the 10th day of floating culture. a,b: sarcomeric myosin, c,d: troponin I, e,f: α -actinin, g,h: ANP.

Please amend the Figure 7A description at page 19, lines 17-25 as follows:

Figure 7A shows the differentiated cardiomyocytes using co-culture system with feeder cells. ES cells (R1) were seeded on ST2 feeder cells, and myocardial differentiation was investigated by ~~immunocyto-chemical~~ immunocytochemical staining using anti-sarcomeric myosin antibodies (MF20) on the 8th day after seeding (n>5). *p<0.01 relative to Noggin (-) group.

Please replace paragraph [0052] with the following replacement paragraph:

In the pre-differentiation stage, the substance ~~that inhibits~~ that inhibits BMP signaling in pluripotent stem cells is applied beginning 1 to 2 days before or preferably 3 days or more before the EBs are formed to induce differentiation.